UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
Antimicrobials Division

January 31, 2003

MEMORANDUM:

Subject: Efficacy Review EPA Reg.No. 70271-13 *Pure Bright Germicidal Ultra Bleach*
DP Barcode 285722
Case No. 068140

From: Nancy Whyte, Microbiologist NOW
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

To: Robert Brennis/Wanda Mitchell
Regulatory Management Branch I
Antimicrobials Division (7510C)

Thru: Emily Mitchell, M.S., Team Leader Emily Mitchell 2/13/03
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Thru: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: KIK International. Inc.
33 McIntosh Blvd.
Concord, Ont. CN

Formulation Label: **% by wt.**

Active Ingredient(s)

Sodium hypochlorite.....6.0%
Other Ingredients.....94.0%
Total.....100.0%

I. Background:

The product, Pure Bright Germicidal Ultra Bleach (EPA Reg. No. 70271-13), is an EPA-approved disinfectant (bactericide, tuberculocide, fungicide, and virucide) and sanitizer for use

on hard, non-porous surfaces in commercial, institutional, hospital, and household environments. The applicant requested an amendment to the registration of this product to add claims for effectiveness against additional microorganisms, specifically Adenovirus type 2, Canine Parvovirus, Cytomegalovirus, Feline Panleukopenia virus (Parvovirus), Hepatitis A virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Influenza A virus, Poliovirus type 1, Respiratory syncytial virus, Rhinovirus type 37, Rotavirus, *Shigella dysenteriae, Escherichia coli* O157:H7, and *Streptococcus pyogenes.* Although the product was already approved as a hospital disinfectant and fungicide, additional efficacy studies (conducted at a 5-minute contact time) were provided using *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella choleraesuis*, and *Trichophyton mentagrophytes.* All studies were conducted at AppTec ATS, located at 2540 Executive Drive in St. Paul, Minnesota 55120.

This data package contained EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-35 (Data Matrix), fifteen studies (MRID Nos. 457600-01 through 457600-15), Statements of No Data Confidentiality Claims for all fifteen studies, and the proposed label. The initial review of the submitted data was conducted by the contractor DynCorp with concurrence with its recommendations by the Product Science Branch of the Antimicrobial Division.

II. Use Directions:

The product is designed to be used for disinfecting hard, non-porous, non-food contact surfaces such as floors, walls, and other inanimate surfaces not in direct contact with food. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant on non-food contact surfaces: Pre-wash surfaces and rinse. Mix 3/4 cup of bleach per gallon of water (a 1:22.33 dilution). Spray, rinse, or wipe surface with bleach solution. Let stand for 10 minutes. Drain and air dry. The label also provided these directions for disinfecting hard, non-porous non-food contact surfaces, using rinse and immersion methods:

> Rinse method - Prepare a use solution by mixing 13 ounces of the product with 10 gallons of water (a 1:99.46 dilution). Clean equipment surfaces. Prior to use, rinse all surfaces thoroughly with the use solution. Allow the solution to maintain contact for at least 10 minutes. Do not rinse with water after treatment and do not soak equipment overnight.
>
> Immersion method - Prepare a use solution by mixing 13 ounces of the product with 10 gallons of water (a 1:99.46 dilution). Clean equipment surfaces. Prior to use, immerse equipment in the use solution for at least 10 minutes. Allow surfaces to drain. Do not rinse with water after treatment.

III. Agency Standards for Proposed Change

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different batches, one of which is at least 60 days old, against *Salmonella choleraesuis* ATCC 10708, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 15442. To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required to provide

effectiveness at the 95% confidence level. The above Agency standards are presented in DIS/TSS-1.

Effectiveness of disinfectants against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, but not including viruses, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different batches. To support products labeled as "disinfectants" for specific microorganisms (other than those microorganisms named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are also presented in DIS/TSS-1.

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products Test (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different batches must be employed in the test. Killing of the specific pathogenic fungi, e.g., *Trichophyton mentagrophytes* on all carriers is required. These Agency standards are presented in DIS/TSS-6.

IV. Summary of Submitted Study

1. MRID 457600-01 "AOAC Use-Dilution Method" for Pure Bright Germicidal Ultra Bleach, by David R. Rottjakob. Study conducted at AppTec ATS. Study completion date – July 24, 2002.

This study was conducted against *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, and *Salmonella choleraesuis* ATCC 10708. Three lots (Lot Nos. 13302, 12902, and 08402 (≥60 days old)) of the product, *Pure Bright Germicidal Ultra Bleach*, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. One lot of the product (i.e., Lot No. 08402) was at least 60 days old at the time of testing. Sixty (60) stainless steel penicylinders were tested. The use solution was prepared as a 1:22.33 dilution, using filter sterilized tap water. Sterile

carriers were immersed for 15 minutes in a 48-54 hour old broth culture of the test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers then were dried on filter paper in a sterile Petri dish at 35-37°C for 40 minutes. For each use solution, 60 contaminated carriers were individually transferred to individual tubes containing 10 mL of the use solution and exposed for 5 minutes at 20±1°C. Following exposure, each exposed carrier was then transferred at identical staggered intervals to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. The neutralized subcultures were incubated for 48±4 hours (for studies conducted on 6/24/02, the carriers were incubated for 44.5 hours) at 36°C, then examined for the presence or absence of visible growth. Controls included purity, sterility, viability, neutralization confirmation, and carrier population.

Note: The applicant requested that the protocol be amended to include a retest of the three lots of Pure Bright Germicidal Ultra Bleach against *Pseudomonas aeruginosa*, due to apparent product efficacy failure.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 457600-02 "Fungicidal Use-Dilution Method" for Pure Bright Germicidal Ultra Bleach, by David Rottjakob. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 13302 and 12902) of the product were tested using the AOAC Use-Dilution Method (modified for fungi) as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. Ten (10) stainless steel penicylinders carriers were tested. The use solution was prepared by adding 6.0 mL of the product and 128.0 mL of filter sterilized tap water (a 1:22.33 dilution). Sterile carriers were immersed for 15 minutes in a 10-15 day old conidial suspension of the test organism, at a ratio of 1 carrier per 1.0 mL suspension. The carriers then were dried at 36°C for 40 minutes. For each use solution, 10 contaminated carriers were individually transferred to individual tubes containing 10 mL of the use solution and exposed for 5 minutes at 20°C. Following exposure, each exposed carrier was then transferred at identical staggered intervals to 10 mL of Sabouraud Dextrose Agar with 0.1% sodium thiosulfate for primary subculture. The carriers then were transferred into secondary subculture tubes of 10 mL of Sabouraud Dextrose Agar with 0.07% Lecithin and 0.5% Tween 80 between 30-60 minutes following the first transfer. The neutralized subcultures were incubated for 10 days at 25-30°C, and the agar plate subcultures were incubated for 44-76 hours at 25-30°C. The subcultures then were examined for the presence or absence of visible growth. Controls included purity, sterility, viability, neutralization confirmation, and carrier population.

Note: The MRID [see page 6 of the MRID] appears to incorrectly identify the Study Director as Andrea J. Mesaros. David Rottjakob signed the Good Laboratory Practice Statement [page 3].

3. MRID 457600-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 18, 2002.

This study was conducted against Adenovirus type 2 ATCC VR-846, Strain Adenoid 6) using A549 cells (human lung carcinoma; obtained from ViroMed Laboratories, Inc., Minneapolis, MN) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, were tested using AppTec ATS Protocol DAC02042602 AD2 (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films

of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20°C in a relative humidity of 33% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. A549 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

4. MRID 457600-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 18, 2002.

This study was conducted against Canine Parvovirus ATCC VR-2017, Cornell strain,using A-72 cells (canine tumor cells; obtained from ViroMed Laboratories, Inc., Minneapolis, MN) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, *Pure Bright Germicidal Ultra Bleach*, were tested using AppTec ATS Protocol DAC02042602. CPV (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20.1°C in a relative humidity of 54% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B . A-72 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed using swine red blood cells at 2-8°C. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

5. MRID 457600-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – July 8, 2002.

This study was conducted against Cytomegalovirus ,ATCC VR-538, Strain AD-169 using MRC-5 cells (human embryonic lung cells; obtained from ViroMed Laboratories, Inc., Minneapolis, MN) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02042602.CMV (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20°C in a relative humidity of 39% for 20 minutes. For each batch of disinfectant, separate dried virus films were

exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B . MRC-5 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 19 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

6. MRID 457600-06 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – August 27, 2002.

This study was conducted against Feline Panleukopenia virus ATCC VR-648, Strain Philips-Roxane, using CRFK cells (feline kidney cells; obtained from ViroMed Laboratories, Inc., Minneapolis, MN) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using AppTec ATS Protocol DAC02042602.FPV (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 15°C in a relative humidity of 60% for 25 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 22°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B (Fungizone). CRFK cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 14 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed using swine red blood cells at 2-8°C. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: The applicant provided the data for a failed trial. In that trial, the recoverable dried virus control titer of ≥ 4 $\log_{10}$ was not achieved. Thus, the test was invalid. These data were not used to evaluate efficacy of the test product. See Attachment I of the MRID study.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

7. MRID 457600-07 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – July 8, 2002.

This study was conducted against Hepatitis A virus ,ATCC VR-1358, strain HM-175, using FRhK-4 cells (obtained from ViroMed Laboratories, Inc., Minneapolis, MN) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02042602.HAV (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product

to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20.1°C in a relative humidity of 57% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 20°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B (Fungizone), and 2.0 mM L-glutamine. FRhK-4 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 13 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

8. MRID 457600-08 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against Herpes simplex virus type 1 ATCC VR-733, strain F(1) using rabbit kidney cells (obtained from ViroMed Laboratories, Inc., Minneapolis, MN as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using AppTec ATS Protocol DAC02051302.HSV1 (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.1°C in a relative humidity of 57% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 20°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B . Rabbit kidney cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

9. MRID 457600-09 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against Herpes simplex virus type 2 ATCC VR-734, strain G, using rabbit kidney cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02042602.HSV2 (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.1°C in a relative humidity of

54% for 25 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B Rabbit kidney cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

10. MRID 457600-10 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 18, 2002.

This study was conducted against Influenza A virus, strain Hong Kong ATCC VR-544, using Rhesus monkey kidney cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02042602.FLUA (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 25.0°C in a relative humidity of 39.1% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 22°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B using Vero cells (obtained from ViroMed Laboratories, Inc., Minneapolis, MN as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02051302.POL (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.1°C in a relative humidity of 56% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Vero cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.
Note: ATCC does not currently offer a strain "ATCC VR-1000" for sale. ATCC codifies the "Brunhilde" strain of Poliovirus type 1 as ATCC VR-58.

11. MRID 457600-11 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against Poliovirus type 1, strain Brunhilde (ATCC VR-1000) using Vero cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using AppTec ATS Protocol DAC02051302.POL (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.1°C in a relative humidity of 56% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B (Fungizone). Vero cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.
Note: ATCC does not currently offer a strain "ATCC VR-1000" for sale. ATCC codifies the "Brunhilde" strain of Poliovirus type 1 as ATCC VR-58.

12. MRID 457600-12 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 18, 2002.

This study was conducted against Respiratory syncytial virus, strain Long ATCC VR-26, using Hep-2 cells (human larynx carcinoma; obtained from ViroMed Laboratories, Inc., Minneapolis, MN as the host system. Two lots (Lot Nos. 13302 and 12902) of the product were tested using AppTec ATS Protocol DAC02042602.RSV (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.2°C in a relative humidity of 57% for 25 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 22°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 1.0 mM L-glutamine. Hep-2 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

13. MRID 457600-13 "Virucidal Efficacy of a Disinfectant for Use on Inanimate

Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 18, 2002.

This study was conducted against Rhinovirus type 37, strain 151-1 ATCC VR-1147 using MRC-5 cells (human embryonic lung cells; obtained from ViroMed Laboratories, Inc., Minneapolis, MN as the host system. Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using AppTec ATS Protocol DAC02042602.R37 (copy not provided). Fetal bovine serum, at a concentration of 1%, was present in the virus stock. The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 9.9°C in a relative humidity of 52% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 21°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B . MRC-5 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

14. MRID 457600-14 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Pure Bright Germicidal Ultra Bleach, by Mary J. Miller. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against Rotavirus (strain WA; obtained from the University of Ottawa, Ontario, Canada) using MA-104 cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. The stock virus culture contained no organic soil load. Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using AppTec ATS Protocol DAC02042602.ROT (copy not provided). The use solution was prepared by adding 1.0 mL of product to 21.33 mL of filter sterilized tap water (a 1:22.33 dilution). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20.2°C in a relative humidity of 63% for 20 minutes. For each batch of disinfectant, separate dried virus films were exposed to 2 mL of the use solution for 5 minutes at 20°C. Following exposure, the plates were scraped, the virus-disinfectant mixture was passed through a Sephadex column to detoxify, and titered by serial dilution in serum-free Eagles minimal essential medium (E-MEM) supplemented with 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B (Fungizone), 0.5 µg/mL trypsin, and 2.0 mM L-glutamine. MA-104 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

15. MRID 457600-15 "AOAC Use-Dilution Method" Pure Bright Germicidal Ultra Bleach, by David R. Rottjakob. Study conducted at AppTec ATS. Study completion date – June 28, 2002.

This study was conducted against *Shigella dysenteriae* (ATCC 13313), *Escherichia coli* O157:H7 (ATCC 35150), and *Streptococcus pyogenes* (ATCC 12344). Two lots (Lot Nos. 13302 and 12902) of the product, Pure Bright Germicidal Ultra Bleach, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The use solution was made by using 45 mL of the product and 959.85 mL of filter sterilized tap water (a 1:22.33 dilution). Ten stainless steel penicylinders carriers were tested for each lot with each organism. No organic soil load was used. Sterile carriers were immersed for 15 minutes in a 48-54 hour old broth culture of the test organism, at a ratio of 1 carrier per 1.0 mL broth. The carriers then were dried on filter paper in a sterile Petri dish at 35-37°C for 40 minutes. For each use solution, 10 contaminated carriers were individually transferred to individual tubes containing 10 mL of the use solution and exposed for 5 minutes at 20±1°C. Following exposure, each exposed carrier was then transferred at identical staggered intervals to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. The neutralized subcultures were incubated for 51 hours at 36°C, then examined for the presence or absence of visible growth. Controls included purity, sterility, viability, neutralization confirmation, and carrier population.

Summary of results of studies:

MRID Number	Organism	No +/Total No. Tested			Carrier Population (CFU/ carrier)
		Lot No. 13302	Lot No. 12902	Lot No. 08402	
457600-01	*Pseudomonas aeruginosa*				
	Test Date: 6/5/02	3/60	7/60	11/60	5.3×10^7
	Test Date: 6/24/02	7/60	2/60	3/60	7.2×10^5
	Test Date: 6/24/02 (retest)	1/60	1/60	1/60	7.7×10^4
	Staphylococcus aureus 6/4/02	1/60	0/60	0/60	3.3×10^6
	Salmonella choleraesuis 6/5/02	0/60	0/60	0/60	7.8×10^4

MRID Number	Organism	No +/Total No. Tested		Carrier Population (CFU/ carrier)
		Lot No. 13302	Lot No. 12902	

457600-02	*Trichophyton mentagrophytes*	1°=0/10 2°=0/10	1°=0/10 2°=0/10	6.0×10^5

MRID	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
			Lot No. 13302	Lot No. 12902	
457600-03	Adenovirus type 2	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{7.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-04	Canine Parvovirus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-05	Cytomegalo-virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-06	Feline Panleukopenia virus (Parvovirus)	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-07	Hepatitis A virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-08	Herpes simplex virus 1	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-09	Herpes simplex virus 2	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-10	Influenza A virus, strain Hong Kong	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-11	Poliovirus type 1	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{7.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
			Lot No. 13302	Lot No. 12902	
457600-12	Respiratory syncytial virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-13	Rhinovirus type 37	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457600-14	Rotavirus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	No +/Total No. Tested		Carrier Population (CFU/ carrier)
		Lot No. 13302	Lot No. 12902	
457600-15	*Shigella dysenteriae*	0/10	0/10	6.0×10^6
	Escherichia coli O157:H7	0/10	0/10	8.5×10^6
	Streptococcus pyogenes	0/10	0/10	6.6×10^5

V. Labeling:

1. The proposed label contains 22 pages of directions. This product is designed to be used in households as well as in commercial institutions and establishments. It is unrealistic to expect that the ordinary household resident would be able to prepare correctly the dilutions necessary to disinfect some areas using the conflicting directions given in the proposed label. Some of the directions are confusing, others are not consistent. In the instructions for preparation of dilutions yielding a certain parts per million(ppm) solution, some of the ppm numbers given are incorrect.

2. The label must use an asterisk attaching the claims of the product as "bactericidal", "fungicidal", and use as "virucide" to a list of specific organisms for which efficacy testing has been done, and for which the product has demonstrated effectiveness at the specific dilution and time exposure listed in the directions. The list of organisms appears on Page 3 of the label, but is not properly referenced by an asterisk on either page.

3. The label directions [page 12 of 22] for preparing a disinfecting rinse using the immersion method state to "immerse in the *disinfecting* solution for at least 10 minutes and allow the *sanitizer* to drain." The Agency considers sanitizers and disinfectants to

be different classes of antimicrobial products. Separate directions must be given for both disinfection and sanitization. This language appeared on both the proposed label and the last accepted label (dated May 15, 2002).

4. The label has multiple sets of directions for preparing a solution of the product for use as a disinfectant. As shown in the following table, these directions are inconsistent with regard to the amount of product per amount of water needed for disinfection:

Application	Use Solution	Dilution
Disinfecting walls, floors, and other hard inanimate surfaces not in direct contact with food [page 5 of 22]	3/4 cup of the product per gallon of water	1:22.33
Disinfecting bathrooms [page 7 of 22]	1.5 cups of the product per 2 gallons of water	1:22.33

3. There are no specific directions on the label for use of the product against pathogenic fungi. With the exception of HIV, there are no specific directions on the label for use of the product as a virucide.

VI. Comments and Recommendations:

1. The submitted efficacy data support the use of the product, Pure Bright Germicidal Ultra Bleach, as a disinfectant with bactericidal activity when tested against *Shigella dysenteriae*, *Escherichia coli* O157:H7, and *Streptococcus pyogenes* on hard, non-porous surfaces for a contact time of 5 minutes at a 1:22.33 dilution

2. Efficacy against *Trichophyton mentagrophytes*, Adenovirus type 2, Canine Parvovirus, Cytomegalovirus, Feline Panleukopenia virus, Hepatitis A virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Influenza A Hong Kong/8/68 virus, Poliovirus type 1, Respiratory syncytial virus, Rhinovirus type 37, and Rotavirus was demonstrated using a 1:22.33 dilution of Pure Bright Germicidal Ultra Bleach for 5 minutes.

3. The submitted efficacy data support the use of the product, Pure Bright Germicidal Ultra Bleach, as a disinfectant with bactericidal activity when tested against *Staphylococcus aureus*, *Salmonella choleraesuis,* and *Pseudomonas aeruginosa* on hard, non-porous surfaces for a contact time of 5 minutes at a 1:22.33 dilution.

4. The label must be revised to correct the deficiencies noted in the Labeling section.